Follicular Cell Implantation: An Emerging Cell Therapy for Hair Loss

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ABSTRACT

The cells of the adult follicular dermal papilla retain a powerful hair-inductive capacity acquired during embryonic hair morphogenesis. This inductive capacity is the basis of a cell therapy called *follicular cell implantation* in which dermal papilla cells from a small number of donor follicles are expanded in culture and then implanted into a bald region of scalp. In this manner, many new follicles can be formed from the cells of a few.

KEYWORDS: Hair, regenerative medicine, hair cloning, dermal papilla, cell therapy

The dermal papilla (DP) is comprised of fibroblast-like cells that are almost unique among adult mammalian cells in that they possess an inductive property that they acquired in the embryo during hair morphogenesis, a property they maintain throughout adult life. This property is the basis of an emerging cell therapy called *follicular cell implantation* (FCI)[1] in which dermal papilla cells taken from a few follicles are expanded in culture and then implanted into the skin to induce the formation of many new follicles.

The idea behind the DP as a cell therapy to treat hair loss is not new.[2] The DP was long assumed to play a critical role in follicle morphogenesis,[3] and the first direct evidence was the demonstration in the 1960s that transplanted dermal papillae could induce the formation of new follicles. Borrowing from experiments with avian feather papillae, Cohen[4] transplanted dermal papillae into rat ear and observed epidermal downgrowths, the beginnings of new follicles. Oliver[5] placed dermal papillae into the base of whisker follicles that were truncated and thereby rendered unable to regenerate, and the transplanted papillae were able to induce the complete regrowth of those follicles. These experiments first revealed the remarkable hair-inductive power of the adult follicle dermal papilla.

Although the concept behind FCI is simple, four decades have passed since the Cohen and Oliver experiments without the introduction of a clinically useful treatment. Here we describe the conceptual and experimental framework behind this therapy, the technical barriers to its development, and how recent advances have brought the technology forward such that FCI may become available in the coming years.

BIOLOGY OF THE HAIR FOLLICLE

The hair follicle mainly consists of an epithelial component and a mesenchymal component. The epithelial component is contiguous with interfollicular epidermis and is comprised of specialized keratinocytes (see Fig. 1). Epidermal stem cells reside in the bulge region of the follicle,[6] a portion of the outer root sheath near the attachment point for the arrector pili muscle. The progeny of stem cells migrate down the outer root sheath[7] to the matrix, and from within the matrix they differentiate into the various concentric layers that comprise the inner root sheath and hair shaft. The dermal papilla is a ball of fibroblast-like cells in the lower part of the follicle and is connected to the dermal sheath through a short stalk at its base. Keratinocyte

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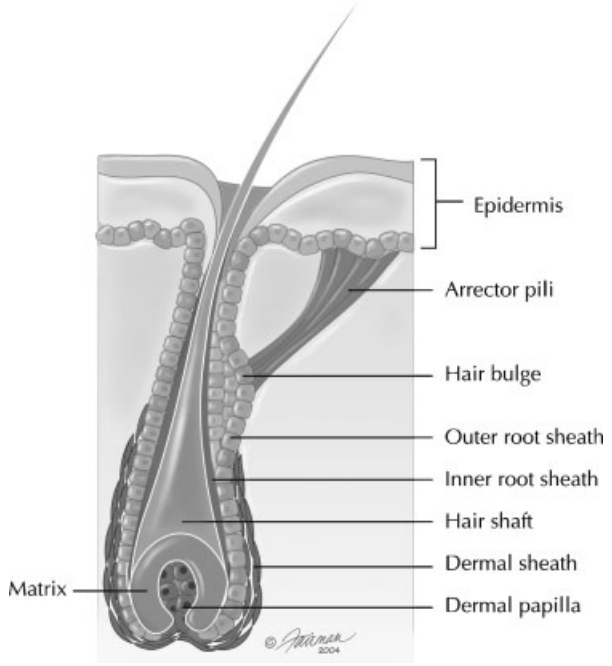

Figure 1 Anatomy of a hair follicle.

migration and differentiation within the follicle are directed in large part by the dermal papilla during morphogenesis in the embryo and during the hair cycle in the adult.

Hair follicle development in the embryo arises through a complex interaction between the embryonic ectoderm and its underlying mesoderm and is mediated by soluble factors secreted by both cell types (reviewed by Hardy[8] and Millar[9]). The first morphologically recognizable step is the appearance of epidermal placodes, which are regularly spaced thickenings in the embryonic epithelium. Clusters or condensates of dermal cells then form beneath the placodes. Peglike downgrowths of epidermis begin to form from the placodes, and they push the dermal condensates down as they grow into the embryonic dermis. Further maturation ensues as the developing follicles grow deeper into the dermis. The epidermal component begins to differentiate into the various follicular epithelial substructures such as the outer root sheath, inner root sheath, and matrix. In the meantime, the dermal condensate forms the dermal papilla as it becomes more compact, and it becomes almost completely enveloped by the developing follicular epithelium. A portion of the condensate forms the dermal sheath. An increasingly detailed picture of the molecular mechanisms that regulate hair follicle formation (reviewed in Millar[9] and Botchkarev and Paus[10]) has revealed a variety of intercellular signaling pathways that are active during follicle morphogenesis, many of which are the same pathways that are used repeatedly throughout mammalian development.[11]

Throughout adult life, the follicle goes through a continuous hair cycle of growth, regression, and rest (reviewed in Stenn and Paus[12] and Botchkarev and Paus[10]). Anagen, the growth phase, is characterized by rapid hair growth supported by extensive proliferation of keratinocytes in the follicular epithelium. During catagen, the lower two thirds of the follicle undergo involution and degradation through apoptosis. Then the follicle enters the resting phase, telogen, in which no hair growth occurs. To reenter anagen, a signal that probably originates from the dermal papilla triggers stem cells within the bulge to undergo a burst of cell proliferation and the progeny migrate out to repopulate the follicular epithelium.[6] Because the cell migrations and the interactions between the dermal papilla and follicular epithelium in the adult hair cycle are similar to those in embryonic hair morphogenesis, the two processes are thought to share signaling pathways in common.[10]

The Dermal Papilla and Sheath in Hair Growth and Development

Throughout both hair morphogenesis and the hair cycle, the dermal papilla plays an important role in signaling the epidermis and thereby influencing cell proliferation and differentiation. Several observations have expanded upon the transplantation experiments of Cohen and Oliver, which first demonstrated the inductive role of the dermal papilla. For example, transplantation of a rat whisker papilla to the ear skin was shown to induce a whiskerlike follicle, demonstrating that the DP specifies the type of hair.[13] The papilla and lower sheath were shown to be essentially equivalent in their hair-inductive capability. The lower portion of the dermal sheath was shown through transplantation to have hair-inductive properties like the papilla,[14] and if the dermal papilla was removed it could be regenerated by cells from the dermal sheath.[15] Indeed, dermal papilla and sheath cells (DP/S cells) move back and forth between the two compartments during the hair cycle[16] and therefore appear to be interchangeable both physically as well as functionally. In remarkable demonstrations of the power of the DP to induce hair formation, transplanted DP and cultured DP cells were shown to induce hair formation even in hairless regions of adult rat skin such as scrotum[17] and foot pad.[18]

The examples cited above are a part of a large body of research that dates back more than 40 years. Although much of the research has been performed using rodent follicles, many of those experiments have been repeated using human tissue,[19] and trans-species (human and rodent) recombinations have been shown to work in a variety of hair induction assays[20,21] (J. Qiao, E. Philips, J. Teumer, data not shown). Thus, the research that provides the experimental and conceptual underpinnings of FCI has proven to extend to human hair induction.

HAIR LOSS AND CURRENT TREATMENTS

Hair loss is a common problem with multiple causes that range from hormone sensitivity to autoimmunity. Androgenetic alopecia, often called male pattern baldness, is the most common form of hair loss in men, and because it affects as many as 50% of men as they age[22] it will be the primary form of hair loss targeted for treatment by FCI. In androgenetic alopecia, hair loss is caused by a sensitivity of hair follicles in the top of the scalp to the androgen 5α-dihydrotestosterone (DHT). DHT causes those follicles to undergo a progressive miniaturization to the point where they no longer produce a clinically apparent hair shaft. The cells affected by DHT are the dermal papilla cells, which cease growing and lose their ability to direct hair growth.[23]

The negative impact of hair loss on self-image causes many patients to seek treatment. Clinically proven pharmaceutical treatments for male pattern hair loss are topical minoxidil solution[24] and oral finasteride.[25] Minoxidil and finasteride are reasonably effective in slowing hair loss and causing regrowth in many patients, but the effects are limited and only last while the patient uses the drug. Because of the drawbacks to these drug-based therapies, some patients opt for more permanent surgical treatments.

The major surgical option available for hair restoration is autologous hair transplantation, in which follicles are removed from a nonbalding region of the scalp and transplanted to the balding region. Hair transplantation for male pattern baldness was first performed by Orentreich,[26] who observed that transplanted follicles did not undergo progressive miniaturization and were therefore not androgen-sensitive like other follicles in the bald region. This phenomenon was termed *donor dominance*,[26] indicating the donor follicle phenotype of androgen insensitivity is maintained after transplantation and the mechanism of androgenetic alopecia is therefore determined by the follicle type rather than by the scalp region in which the follicle resides. Because donor follicles do not undergo miniaturization, hair transplantation is a permanent treatment for hair loss that lasts until the onset of senescent alopecia late in life.

The early hair transplant procedures employed large, round grafts that were not esthetically satisfactory for most patients. Today, the state-of-the-art technique is follicular unit transplantation, first described by Limmer,[27] which uses follicular unit grafts of one to four hairs that are dissected with a stereomicroscope from a long strip of donor scalp. When performed by a skilled hair transplant surgeon, these transplants yield excellent results that are virtually undetectable. Transplantation is a "one-for-one" movement of follicles from one place to another on the scalp and there is no regeneration of hair in the donor region. The treatment is therefore limited by the amount of donor hair available, which is usually not enough to completely cover the bald region of most patients. Also, despite the considerable skill of the surgeon, the maximum hair density achievable is never the prebalding, youthful density. Other drawbacks include the donor site scar, which can be unacceptably wide in some patients and is of special concern for patients who wear their hair short. In addition, although the modern transplant procedure is noninvasive, relatively painless, and associated with a quick recovery, many people are unwilling to undergo what they see as major surgery.

FOLLICULAR CELL IMPLANTATION

All of the existing medical or surgical therapies available for hair restoration have drawbacks and there is intense effort to develop improvements. Follicular cell implantation (FCI) is an alternative treatment that unlike drugs would be permanent and unlike transplantation would not be limited by the quantity of donor hair. The ability to expand cells in culture would provide enough cells to induce a plentiful number of new follicles from a small number of donor ones. FCI has often been referred to by the misnomers *hair cloning* or *hair multiplication*. These terms are misleading and fail to accurately describe what is meant by *follicular cell implantation*. In FCI, new follicles are induced by the cultured DP cells in conjunction with existing epidermis in the scalp, so that new follicles are a combination of the implanted cells and target epidermis. DP cells are not cloned, nor are whole follicles multiplied, although the idea that many new follicles are formed is correct.

The first versions of FCI are likely to be autologous, in which the patient's own cells are used. A model procedure for autologous FCI is outlined in Fig. 2. A piece of the patient's nonbalding scalp would be removed by a physician in an outpatient clinic. The amount of donor hair required will be much smaller than the large donor strips of up to several thousand follicles used for transplantation. The scalp tissue will be sent to a laboratory where the follicular cells would be isolated from the tissue and expanded in culture. Because the donating scalp will contain androgen-insensitive follicles, the DP cells will be "donor-dominant" just as in transplanted follicles. After the period of culture expansion the cells would be harvested and then sent back to the clinic for implantation. There are two major technical challenges inherent in the procedure: (1) the expansion of cells in culture and (2) the implantation of cells into the patient's scalp to effect hair restoration.

Expansion of Dermal Papilla/Sheath Cells in Culture

In most examples of cell therapy, cells are expanded in culture to grow enough cells for regeneration of the tissue or organ. An important part of the success of the

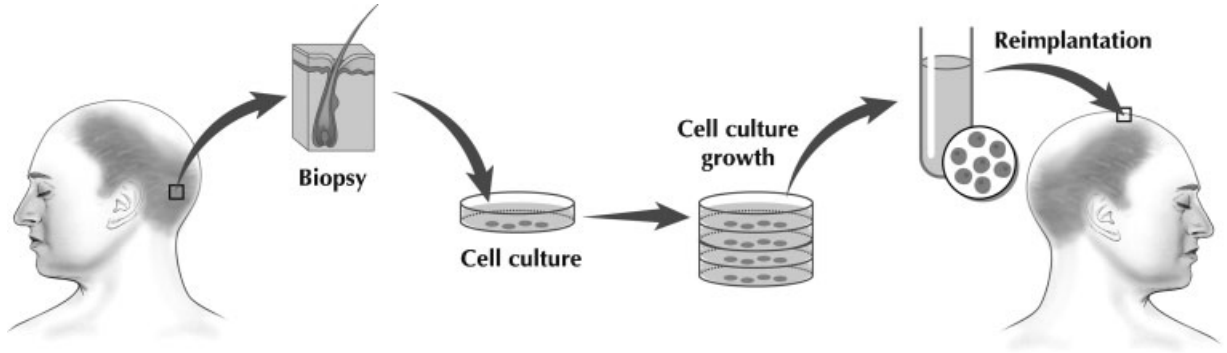

Figure 2 Process of Follicular Cell Implantation.

therapy is the culture system, which must support cell multiplication and also maintain cell function. This challenge is an important one and remains a major block in the development of cell therapies for many cell types. Loss of the functional phenotype during cell culture is usually a permanent loss that cannot be regained upon implantation in vivo.

Dermal papilla (DP) cells were first shown to be culturable in vitro many years ago,[2,28,29] but the culture systems only allowed for limited expansion before the hair-inductive capability was lost. Like many other cultured cell types, after a few population doublings in culture, the critical phenotype was lost. The inability to expand hair-inductive DP cells in culture was probably the most important barrier to the development of FCI, because insufficient expansion would not provide enough cells to regenerate a significant number of new follicles and the therapy would not provide an advantage over existing hair transplantation.

Two recent advances in cell culture of DP cells, in part a direct result of other advances in the understanding of hair biology, have resulted in the development of culture conditions that support significant cell expansion and preserve hair induction. One system employs keratinocytes, the cells that naturally interact with dermal papilla cells in the hair follicle. Yoshizato and colleagues[30,31] showed that coculture of DP cells with epidermal keratinocytes as feeder cells would maintain the DP cells' hair-inductive property. Reynolds and Jahoda[32] also showed that DP cells can be grown in coculture with matrix keratinocytes. Coculture has been used successfully in other culture systems to promote cell growth from the feeder cells through cell–cell contact and secreted soluble factors.

In addition to coculture, Yoshizato and colleagues proved that keratinocyte-conditioned medium can also support DP cell growth,[30] demonstrating that soluble factors are necessary but cell–cell contact is not necessary to maintain the hair-inductive capability. In culture, keratinocytes secrete soluble factors into the medium that work to preserve hair induction, while DP cells grow and divide in culture. The identity of these keratinocyte-generated factor(s) is not known, but they may be the same or similar to the factor(s) that are secreted by keratinocytes during hair morphogenesis.

A second DP cell culture system is based upon the discovery that WNT signaling is active during hair morphogenesis.[33,34] The WNT pathway is active in the epidermal placode during development, and WNT proteins are thought to be part of the signal that triggers the dermal condensate to form.[35] In addition, WNT family members are expressed in adult follicular epithelium,[36,37] suggesting a role for WNT signaling in maintaining the hair-inductive capability of DP cells in the adult follicle. When WNT proteins were provided to mouse DP cells via coculture with WNT overproducing feeder cells, the DP cells could be expanded for several passages and the hair-inductive capability of the cells preserved. In a hair-induction assay, DP cells had retained the ability to direct the formation of new hair.[35]

Implantation

The second challenge to successful FCI is the implantation of cultured cells into the patient's scalp. The primary consideration is that the signaling molecules involved in follicular morphogenesis only act over very short distances, so implanted cells need to be placed in immediate proximity to keratinocytes for meaningful signaling to occur. Another consideration is that the extracellular matrix in the adult scalp is much denser and more collagenous than the matrix surrounding the developing follicle, and it is not known if a nascent follicle will penetrate the adult matrix and grow down into the dermis like it does during normal development. If the new follicle were to remain superficial, it's possible it may not be anchored well enough to withstand damage through normal pulling from brushing or washing.

In a normal follicle, the dermal papilla is a tight cluster of cells with its own extracellular matrix.[38,39] In FCI, a tight structure with a critical cell mass must form to generate a signal sufficient to induce follicle

formation. A loose or unconcentrated group of cells may not send a signal adequate for proper induction. Fortunately, cultured DP cells possess a natural propensity to aggregate in vivo and in vitro.[40,41] If single cell suspensions were implanted, they could spontaneously aggregate in situ. Alternatively, DP cell aggregates could be preformed in vitro and implanted.

Jahoda[13] has shown that the size of a transplanted dermal papilla determines the diameter of the shaft in the induced follicle, and Ibrahim and Wright[42] showed papilla size correlates with hair shaft diameter. To achieve a consistent, esthetically acceptable hair thickness and texture, each individual cell implant will need to contain a constant cell number to generate a follicle of consistent size. In addition, because the angle of hair follicles varies among individuals and in different regions of the scalp, controlling the angle at which the new follicles grow will be important for the clinician to achieve the proper aesthetic result.

Implantation of cells into such a precise location in the manner necessary for hair regeneration has not been done before. Placement of cells in a very shallow position just below the epidermis is technically challenging. No quantitative studies have been published that demonstrate the minimum number of DP cells required for a hair to form. In the published accounts of DP transplantation, cells or DP structures were implanted in a relatively uncontrolled and imprecise manner. Those techniques would have to be improved upon to achieve consistency and efficiency, as the procedure would need to involve many hundreds, perhaps thousands of injections in a single session. This new type of cell implantation may require the development of novel devices that can rapidly and reproducibly implant cells in the proper number and at the proper depth and appropriate angle in the skin.

A likely target for implantation in the scalp is interfollicular epidermis. Oliver[17] and Jahoda and Reynolds[18] demonstrated that new follicles could be induced in hairless skin of rat. Ferraris et al[21] demonstrated that human interfollicular epidermis retains the ability to form hair. These findings demonstrated the dermal papilla is capable of inducing adult nonfollicular epidermis to form hair and were early indications of the plasticity of interfollicular epidermal stem cells. That DP cells can induce hair from interfollicular epidermis is an important proof of principle for follicular cell implantation. A speculative model for interfollicular FCI is outlined in Fig. 3. In this model, implanted cells would aggregate near the epidermis and signal keratinocytes to form a hair peg, which would grow down into the dermis and surround the aggregated DP cells as in embryonic development. Successful interfollicular FCI would mean that hair restoration could be achieved in patients with hair loss due to burns, trauma, or scarring alopecias who otherwise have too little donor hair for traditional transplants.

Another possible route is intrafollicular implantation of cells into preexisting follicles. The targets would be miniaturized follicles that are cosmetically insignificant, and the strategy would be to rejuvenate miniaturized follicles by the insertion of hair-inductive DP cells.

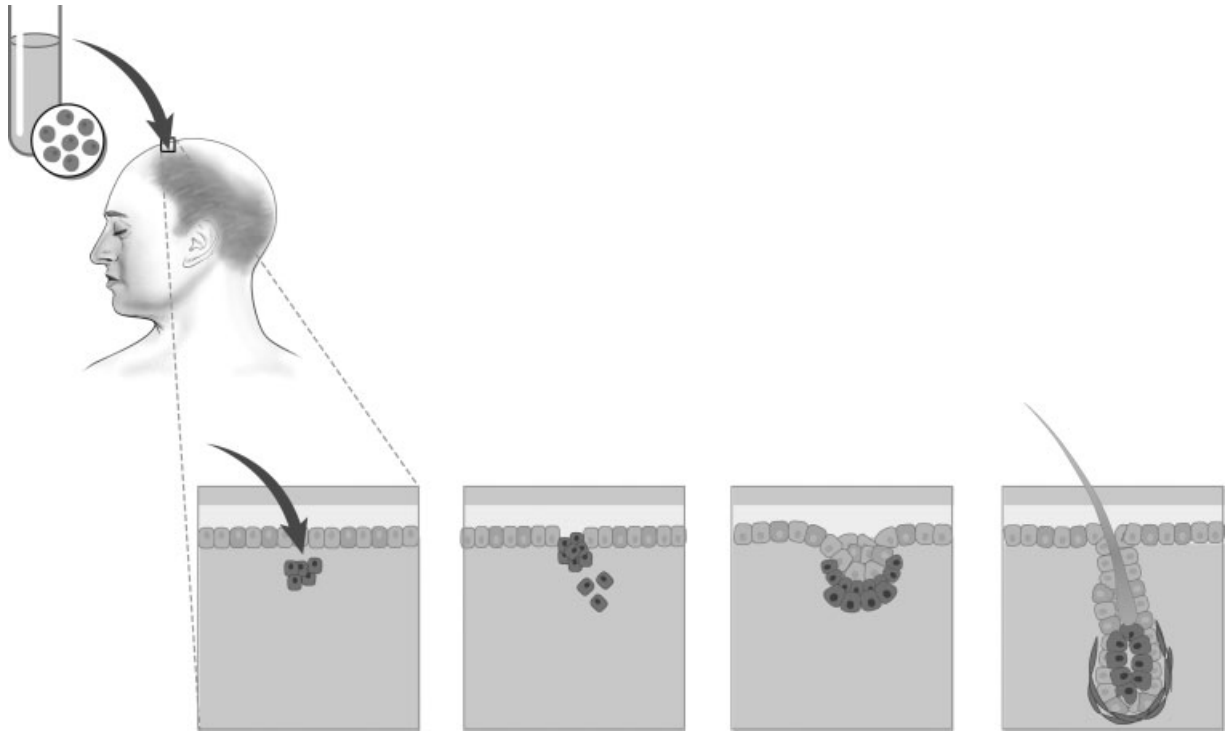

Figure 3 Proposed model of hair induction by interfollicular FCI.

Because the cellular target in androgenetic alopecia is the dermal papilla, providing the follicle with new, androgen-insensitive DP cells might reactivate the follicle to form a normal (terminal) hair. The mechanism of intrafollicular FCI would probably resemble reentry into anagen in the hair cycle, except from a new dermal papilla.

Intrafollicular implantation would take advantage of the semblance of normal follicle structure retained by miniaturized follicles. Rejuvenating or reactivating such preexisting hairs may be easier than forming new ones as in interfollicular FCI. Additionally, these follicles would already be oriented in the proper direction on the patient's scalp, obviating the need for the physician to reconstruct this orientation. McElwee et al[43] have shown that intradermally implanted dermal sheath cells have the ability to incorporate into the dermal papillae of preexisting follicles, creating a chimera between preexisting and implanted cells. Thus, implanted cells can potentially home to and become a part of preexisting dermal papillae in miniaturized follicles. Alternatively, the implanted cells might aggregate to form their own, nascent DP from which to reactivate the miniaturized follicle.

In a variation of FCI, dermal papilla cells could be coimplanted with keratinocytes. The inclusion of keratinocytes could help jump-start the signaling process and also provide a bridge between DP cells and the recipient epidermis. Because the coimplanted keratinocytes would already be in close proximity to implanted DP cells, this technique may allow for a deeper injection that may result in a more firmly anchored new follicle. It may also provide a little room for error in the placement of implants that may otherwise be required to be very shallow and difficult to achieve. A specialized keratinocyte called the germinative epithelium, located in the lower matrix, is thought to contribute to the differentiation of other epithelial cells in the follicle.[32,44,45] The use of these specialized keratinocytes may make coimplantation more efficient in inducing new follicles.

Implantation of mixed suspensions of keratinocytes and dermal cells are known to have the ability to properly sort into epidermis and dermis in a murine graft model and thereby reconstitute skin and hair.[46] This model has proven to be useful for the assay of hair-inductive DP cells of murine and human origin[35,47] (J. Qiao, E. Philips, and J. Teumer, unpublished). A scaled-down mixed implant of cells in FCI could reform hair in a similar manner. The new hair would be comprised of the donor DP plus a follicular epithelium mixture of donor and recipient keratinocytes.

Allogeneic FCI

FCI that used an allogeneic cell donor would be a simpler process than an autologous one because cells from a single donor could be expanded, cryopreserved, and then later used to treat many recipients. Such a procedure would be more streamlined and would not require a biopsy and individualized cell culture for every patient. A follicle resulting from allogeneic FCI would be a chimera of the patient's epidermal cells and the donor DP cells.

The biggest problem with allogeneic transplantation is usually graft rejection by the host. However, study of the hair follicle immune system has led to speculation that it might be possible to transplant allogeneic hair follicle cells without rejection. The hair follicle has been demonstrated to be an immune-privileged site, a host site into which allogeneic tissue can be transplanted without being rejected.[48] Immune privilege within a host hair follicle is not directly relevant to the immune response to transplanted follicular cells, but certain characteristics of cells from immune-privileged sites[49] are relevant to their immunological fate after transplantation. One such characteristic is the absence of expression of molecules necessary to stimulate a host immune reaction.[50] In particular, follicular cells do not express major histocompatibility class (MHC) I or class II molecules, which are necessary for graft rejection by the host immune system.[51]

Reynolds et al demonstrated hair induction by transplanted allogeneic dermal sheath in a human subject.[52] In this experiment, freshly isolated male dermal sheath was transplanted into a female recipient, and new hair was observed after 3 to 5 weeks. When the new follicles were biopsied and examined histologically up to 11 weeks after implantation, none of the follicles showed signs of rejection even though it was demonstrated that the dermal papilla was of donor origin. The absence of donor MHC class II–expressing cells such as endothelial cells or Langerhans cells undoubtedly reduced the possibility of acute rejection, but the cells themselves may avoid acute rejection.

Some cell types such as keratinocytes and fibroblasts that do not express MHC class II or costimulatory molecules such as B7 cannot directly present antigen to T cells and therefore cannot stimulate an acute rejection response.[53,54] Allogeneic keratinocytes and fibroblasts have been transplanted without evidence of acute rejection,[55–57] and keratinocyte allografts have been shown to persist for over 1 year.[57] However, the number of persistent cells was very low compared with the number engrafted,[57] and slower-acting mechanisms of graft rejection may have taken place. Alternatively, as these allogeneic cells were grafted into wounds, the wound healing response may have exerted a negative effect on graft survival. DP/S cells appear to be similar to keratinocytes and fibroblasts in the absence of those molecules necessary for direct antigen presentation, making it likely that allogeneic DP/S cells would behave like keratinocytes or fibroblasts in a cell therapy and would

not be acutely rejected after implantation. In FCI there would be no significant wound healing response, so associated problems with survival would not occur and long-term persistence of the implanted cells would be more likely. Overall, the success of allogeneic dermal sheath grafting and the similarity of DP/S cells to dermal fibroblasts in their expression of MHC molecules suggest that allogeneic FCI may be feasible but will require more investigation.

CONCLUSION

The mesenchyme-derived cells of the adult hair follicle possess the remarkable ability to regenerate hair. An abundance of research demonstrates the ability of dermal papilla and sheath cells to induce new hair formation in a variety of model systems and provides a strong foundation for the concept of FCI as a therapy to treat hair loss.

Regeneration or replacement of tissues and organs remains a significant challenge in medicine. Historically, allogeneic transplantation has been the only treatment option and it remains the only option for most major organs. The shortage of donor organs and the tremendous advances in the understanding of developmental biology, cell biology, and other fields have inspired new therapeutic strategies such as cell therapy. Cell therapy places the burden of regeneration on the innate ability of the implanted cells to perform their natural, preprogrammed role to achieve regeneration in situ. In some cases, such as in epidermis or cartilage, the implanted cells assume their normal structural role in the tissue and assist regeneration as part of the normal wound healing response. In the case of FCI, the DP cells induce keratinocytes to form new hairs in a process that recapitulates embryonic hair morphogenesis. The inductive process sets FCI apart from existing cell therapies and raises the mechanistic complexity from the level of simple replacement to a level of actual regeneration. The strategy of regeneration in situ may provide a means to replace or provide functional repair in more complex organs. Embryonic stem cell technologies may eventually make possible the production in culture of inductive organ progenitor cells that can then be implanted to effect organ regeneration in situ.

In conclusion, the small size and relative accessibility of the hair follicle, the demonstrable hair induction by adult dermal papilla and sheath cells, and the ability to expand them in culture make FCI a feasible therapy that should be available in the near future. Recent research has uncovered some of the mechanisms through which these cells interact with the follicular epithelium to form a follicle, and these mechanisms are shared with other morphogenetic processes that occur during organogenesis.[9,11] Just as basic research on simple systems sheds light on more complex systems, so too may the development of a cell therapy for the relatively simple hair follicle help in the future development of cell therapies for the regeneration of more complex organs.

REFERENCES

1. Cooley J. Follicular cell implantation: an update on "hair follicle cloning". Facial Plast Surg Clin North Am 2004;12: 219–224
2. Jahoda CA, Horne KA, Oliver RF. Induction of hair growth by implantation of cultured dermal papilla cells. Nature 1984; 311:560–562
3. Chase HB. Growth of the hair. Physiol Rev 1954;34:113–126
4. Cohen J. The transplantation of individual rat and guinea-pig whisker papillae. J Embryol Exp Morphol 1961;9:117–127
5. Oliver RF. The experimental induction of whisker growth in the hooded rat by implantation of dermal papillae. J Embryol Exp Morphol 1967;18:43–51
6. Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell 1990;61:1329–1337
7. Oshima H, Rochat A, Kedzia C, Kobayashi K, Barrandon Y. Morphogenesis and renewal of hair follicles from adult multipotent stem cells. Cell 2001;104:233–245
8. Hardy MH. The secret life of the hair follicle. Trends Genet 1992;8:55–61
9. Millar SE. Molecular mechanisms regulating hair follicle development. J Invest Dermatol 2002;118:216–225
10. Botchkarev VA, Paus R. Molecular biology of hair morphogenesis: development and cycling. J Exp Zoolog B Mol Dev Evol 2003;298:164–180
11. Hogan BL. Morphogenesis. Cell 1999;96:225–233
12. Stenn KS, Paus R. Controls of hair follicle cycling. Physiol Rev 2001;81:449–494
13. Jahoda CA. Induction of follicle formation and hair growth by vibrissa dermal papillae implanted into rat ear wounds: vibrissa-type fibres are specified. Development 1992;115: 1103–1109
14. Horne KA, Jahoda CA. Restoration of hair growth by surgical implantation of follicular dermal sheath. Development 1992; 116:563–571
15. Oliver RF. Whisker growth after removal of the dermal papilla and lengths of follicle in the hooded rat. J Embryol Exp Morphol 1966;15:331–347
16. Tobin DJ, Gunin A, Magerl M, Handijski B, Paus R. Plasticity and cytokinetic dynamics of the hair follicle mesenchyme: implications for hair growth control. J Invest Dermatol 2003;120:895–904
17. Oliver RF. The induction of hair follicle formation in the adult hooded rat by vibrissa dermal papillae. J Embryol Exp Morphol 1970;23:219–236
18. Jahoda CA, Reynolds AJ. Dermal-epidermal interactions—follicle-derived cell populations in the study of hair-growth mechanisms. J Invest Dermatol 1993;101(Suppl):33S–38S
19. Jahoda CA, Oliver RF, Reynolds AJ, Forrester JC, Horne KA. Human hair follicle regeneration following amputation and grafting into the nude mouse. J Invest Dermatol 1996; 107:804–807
20. Jahoda CA, Oliver RF, Reynolds AJ, et al. Trans-species hair growth induction by human hair follicle dermal papillae. Exp Dermatol 2001;10:229–237

21. Ferraris C, Bernard BA, Dhouailly D. Adult epidermal keratinocytes are endowed with pilosebaceous forming abilities. Int J Dev Biol 1997;41:491–498
22. Hogan DJ, Chamberlain M. Male pattern baldness. South Med J 2000;93:657–662
23. Randall VA. The biology of androgenetic alopecia. In: Camacho F, Randall VA, Price VH, eds. Hair and Its Disorders: Biology, Pathology and Management. London: Martin Dunitz; 2000:123–136
24. Price VH, Menefee E, Strauss PC. Changes in hair weight and hair count in men with androgenetic alopecia, after application of 5% and 2% topical minoxidil, placebo, or no treatment. J Am Acad Dermatol 1999;41(5 Pt 1):717–721
25. Kaufman KD, Olsen EA, Whiting D, et al. Finasteride in the treatment of men with androgenetic alopecia. Finasteride Male Pattern Hair Loss Study Group. J Am Acad Dermatol 1998;39(4 Pt 1):578–589
26. Orentreich N. Autografts in alopecias and other selected dermatological conditions. Ann N Y Acad Sci 1959;83:463–479
27. Limmer BL. Elliptical donor stereoscopically assisted micrografting as an approach to further refinement in hair transplantation. J Dermatol Surg Oncol 1994;20:789–793
28. Jahoda C, Oliver RF. The growth of vibrissa dermal papilla cells in vitro. Br J Dermatol 1981;105:623–627
29. Messenger AG. The culture of dermal papilla cells from human hair follicles. Br J Dermatol 1984;110:685–689
30. Matsuzaki T, Inamatsu M, Yoshizato K. Hair induction by dermal papilla cells cultured with conditioned medium of keratinocytes. In: Van Neste DJJ, Randall VA, eds. Hair Research for the Next Millenium. New York: Elsevier Science B.V.; 1996:447–451
31. Inamatsu M, Matsuzaki T, Iwanari H, Yoshizato K. Establishment of rat dermal papilla cell lines that sustain the potency to induce hair follicles from afollicular skin. J Invest Dermatol 1998;111:767–775
32. Reynolds AJ, Jahoda CA. Hair matrix germinative epidermal cells confer follicle-inducing capabilities on dermal sheath and high passage papilla cells. Development 1996;122:3085–3094
33. Noramly S, Freeman A, Morgan BA. beta-catenin signaling can initiate feather bud development. Development 1999;126: 3509–3521
34. DasGupta R, Fuchs E. Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. Development 1999;126:4557–4568
35. Kishimoto J, Burgeson RE, Morgan BA. Wnt signaling maintains the hair-inducing activity of the dermal papilla. Genes Dev 2000;14:1181–1185
36. Millar SE, Willert K, Salinas PC, et al. WNT signaling in the control of hair growth and structure. Dev Biol 1999;207:133–149
37. Reddy S, Andl T, Bagasra A, et al. Characterization of Wnt gene expression in developing and postnatal hair follicles and identification of Wnt5a as a target of Sonic hedgehog in hair follicle morphogenesis. Mech Dev 2001;107:69–82
38. Jahoda CA, Mauger A, Bard S, Sengel P. Changes in fibronectin, laminin and type IV collagen distribution relate to basement membrane restructuring during the rat vibrissa follicle hair growth cycle. J Anat 1992;181(Pt 1):47–60
39. Messenger AG, Elliott K, Westgate GE, Gibson WT. Distribution of extracellular matrix molecules in human hair follicles. Ann N Y Acad Sci 1991;642:253–262
40. Messenger AG, Senior HJ, Bleehen SS. The in vitro properties of dermal papilla cell lines established from human hair follicles. Br J Dermatol 1986;114:425–430
41. Jahoda CA, Oliver RF. Vibrissa dermal papilla cell aggregative behaviour in vivo and in vitro. J Embryol Exp Morphol 1984;79:211–224
42. Ibrahim L, Wright EA. A quantitative study of hair growth using mouse and rat vibrissal follicles. I. Dermal papilla volume determines hair volume. J Embryol Exp Morphol 1982;72:209–224
43. McElwee KJ, Kissling S, Wenzel E, Huth A, Hoffmann R. Cultured peribulbar dermal sheath cells can induce hair follicle development and contribute to the dermal sheath and dermal papilla. J Invest Dermatol 2003;121:1267–1275
44. Reynolds AJ, Jahoda CA. Hair follicle stem cells? A distinct germinative epidermal cell population is activated in vitro by the presence of hair dermal papilla cells. J Cell Sci 1991;99(Pt 2):373–385
45. Reynolds AJ, Lawrence CM, Jahoda CA. Human hair follicle germinative epidermal cell culture. J Invest Dermatol 1993; 101:634–638
46. Lichti U, Weinberg WC, Goodman L, et al. In vivo regulation of murine hair growth: insights from grafting defined cell populations onto nude mice. J Invest Dermatol 1993;101(Suppl):124S–129S
47. Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E. Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. Cell 2004; 118:635–648
48. Barker CF, Billingham RE. Immunologically privileged sites. Adv Immunol 1977;25:1–54
49. Streilein JW. Immune privilege as the result of local tissue barriers and immunosuppressive microenvironments. Curr Opin Immunol 1993;5:428–432
50. Streilein JW. Unraveling immune privilege. Science 1995; 270:1158–1159
51. Christoph T, Muller-Rover S, Audring H, et al. The human hair follicle immune system: cellular composition and immune privilege. Br J Dermatol 2000;142:862–873
52. Reynolds AJ, Lawrence C, Cserhalmi-Friedman PB, Christiano AM, Jahoda CA. Trans-gender induction of hair follicles. Nature 1999;402:33–34
53. Laning JC, DeLuca JE, Hardin-Young J. Effects of immunoregulatory cytokines on the immunogenic potential of the cellular components of a bilayered living skin equivalent. Tissue Eng 1999;5:171–181
54. Laning JC, Deluca JE. Isaacs CM, Hardin-Young J. In vitro analysis of CD40–CD154 and CD28–CD80/86 interactions in the primary T-cell response to allogeneic "nonprofessional" antigen presenting cells. Transplantation 2001;71:1467–1474
55. Briscoe DM, Dharnidharka VR, Isaacs C, et al. The allogeneic response to cultured human skin equivalent in the hu-PBL-SCID mouse model of skin rejection. Transplantation 1999;67:1590–1599
56. Falanga V, Margolis D, Alvarez O, et al. Rapid healing of venous ulcers and lack of clinical rejection with an allogeneic cultured human skin equivalent. Human Skin Equivalent Investigators Group. Arch Dermatol 1998;134:293–300
57. Otto WR, Nanchahal J, Lu QL, Boddy N, Dover R. Survival of allogeneic cells in cultured organotypic skin grafts. Plast Reconstr Surg 1995;96:166–176